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# DYNAMICS OF ISOTACHOPHORETIC SEPARATION

## II*. IMPURITIES IN THE ELECTROLYTES AND SAMPLE INJECTION

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## SUMMARY

The behaviour and effects of impurities in the electrophoretic separation of the cations of strong electrolytes for the case when four components are involved in the separation was studied. The separation dynamics were studied for two characteristic cases, namely, the behaviour and effects of impurities present in the terminating electrolyte and those present in the leading electrolyte.

The effect of sample injection on the course of the isotachophoretic separation was studied. Cases corresponding to real experimental conditions were considered, when the sample was mixed in various ways with the leading and terminating electrolytes during the injection.

## INTRODUCTION AND EXPERIMENTAL

The present state of the modelling of the dynamics of electrophoretic separations, the separation conditions, the mathematical model and the method of its solution utilized in this work have been described in detail in Part I[1]. To study the effect of impurities in the electrophoretic separation of cations of strong electrolytes, a four-component separation was considered. The cation of electrolyte 1 is $K^+$ with a mobility $U_1 = 7.618 \cdot 10^{-4}$ cm$^2$ sec$^{-1}$ V$^{-1}$, the cation of electrolyte 2 is $Na^+$ with a mobility $U_2 = 5.192 \cdot 10^{-4}$ cm$^2$ sec$^{-1}$ V$^{-1}$, the cation of electrolyte 3 is $(C_2H_5)_4N^+$ with a mobility $U_3 = 3.420 \cdot 10^{-4}$ cm$^2$ sec$^{-1}$ V$^{-1}$ and the cation of electrolyte 4 is $(C_4H_9)_4H^+$ with a mobility $U_4 = 1.980 \cdot 10^{-4}$ cm$^2$ sec$^{-1}$ V$^{-1}$. The common counter ion is $Cl^-$ with a mobility, $U_R = 7.918 \cdot 10^{-4}$ cm$^2$ sec$^{-1}$ V$^{-1}$; the current density is $3 \cdot 10^{-2}$ A cm$^{-2}$. Two basic cases for the presence of an impurity in the electrolytes were studied, namely the behaviour and effects of impurities present in the terminating or in the leading electrolyte.

When the terminating (leading) electrolyte contains impurities whose mobility is greater (smaller) than the sample mobility, both the isotachophoresis and the displacement electrophoresis principle participate in the separation. If an impurity in

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* For Part I, see ref. 1.

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the terminating (leading) electrolyte has the greatest (smallest) mobility of all the ions present, a true steady state cannot be formed and all the zones formed, as well as the region of the leading (terminating) electrolyte, will be affected.

The same mathematical model and the same method of its solution were used in the study of the effect of the sample injection on the course of the isotachophoretic separation. It is assumed that the method of sample injection does not substantially affect the isotachophoretic separation, *i.e.*, the attainment of the stationary state, but no detailed study of this effect has so far been carried out.

To study the effect of the method of sample injection on the dynamics of the isotachophoretic separation, a system of four components (strong electrolytes) specified above was chosen. The leading electrolyte cation, L, is $K^+$, the cation of sample component A is $Na^+$, the cation of sample component B is $(C_2H_5)_4N^+$ and the cation of the terminating electrolyte, T, is $(C_4H_9)_4H^+$. The common counter ion and the current density are the same as in the previous instance.

Samples are usually injected either through a sampling valve or with a syringe. Therefore, the effects of these two methods of sample injection on the dynamics of the isotachophoretic separation were studied.

## RESULTS

### *Behaviour and effect of an impurity present in the terminating electrolyte*

The separation dynamics in the presence of an impurity in the terminating electrolyte were studied for three characteristic cases, as follows.

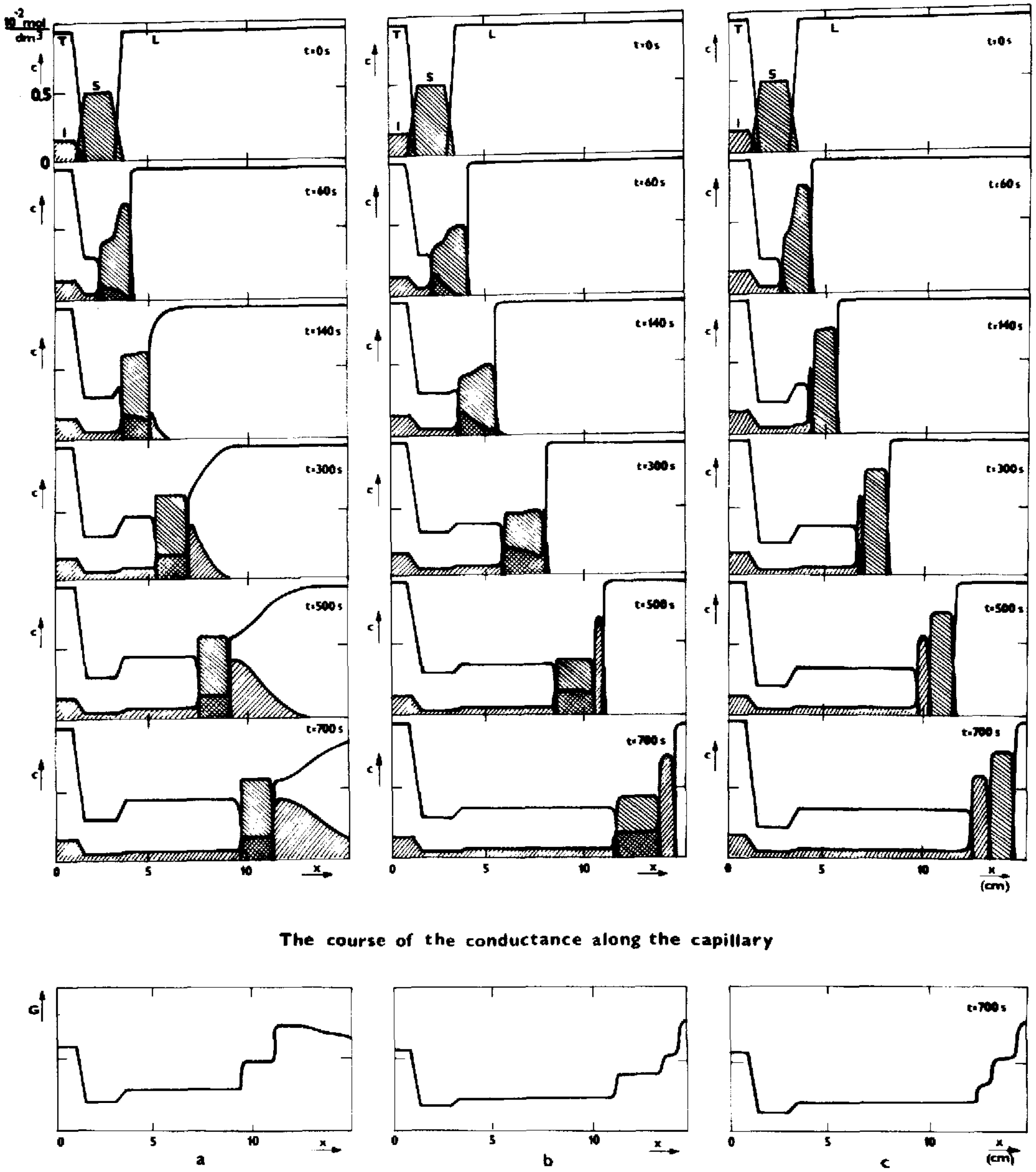
(a) Impurity I is electrolyte 1, leading electrolyte L is electrolyte 2, terminating electrolyte T is electrolyte 4 and sample S is electrolyte 3. It holds for the particular mobilities that $U_I > U_L > U_S > U_T$. The concentration profiles of the components during the separation are shown in Fig. 1a.

During the separation, the mixed zone of the terminating electrolyte with the impurity is preserved and the concentration ratio, $c_T/c_I$, is constant during the whole separation. A new mixed zone is formed of the sample and the impurity, the concentration ratio, $c_S/c_I$, and the mixed zone length being constant. The inpurity gradually penetrates into the leading electrolyte and forms a mixed region in it. Thus the concentration ratio, $c_L/c_I$, varies with time and position in the capillary.

(b) Impurity I is electrolyte 2, leading electrolyte L is electrolyte 1, terminating electrolyte T is electrolyte 4 and sample S is electrolyte 3. It holds for the mobilities that $U_L > U_I > U_S > U_T$. The concentration profiles of the components are given in Fig. 1b.

During the separation, the mixed zone of the terminating electrolyte and the impurity is preserved and the concentration ratio, $c_T/c_I$, is constant during the whole separation. A new mixed zone is formed of the sample and the impurity with the concentration ratio, $c_S/c_I$, and the mixed zone length constant. The impurity gradually forms an independent zone between the sample and the leading electrolyte. The concentration $c_I$ in this zone is constant and the zone length increases with increasing time. The leading electrolyte remains pure during the whole separation.

(c) Impurity I is electrolyte 3, leading electrolyte L is electrolyte 1, terminating electrolyte T is electrolyte 4 and sample S is electrolyte 2. It holds for the mobilities that $U_L > U_S > U_I > U_T$. The concentration profiles of the components during the separation are given in Fig. 1c.



a b c

Fig. 1. Separation dynamics for impurities present in the terminating electrolyte.

During the separation the mixed zone of the terminating electrolyte and the impurity is preserved and the concentration ratio, $c_T/c_I$, is constant during the whole separation. The impurity gradually forms an independent zone between the terminating electrolyte and the sample. The concentration $c_I$ in this zone is constant and the zone length increases with increasing time. The sample and leading electrolyte zones remain pure during the whole separation.

In addition to the concentration profiles, Fig. 1a–c also depict the course of the conductance $G$ along the capillary after 700 sec of the separation. It can be seen that the identification of the composition of the individual zones based solely on the conductivity detector recording may not be reliable. The concentration profile of the common counter ion is not given.

### *Behaviour and effect of an impurity present in the leading electrolyte*

The separation dynamics in the presence of an impurity in the leading electrolyte were studied for the three following characteristic cases:

(a) Impurity I is electrolyte 4, leading electrolyte L is electrolyte 1, terminating electrolyte T is electrolyte 3 and sample S is electrolyte 2. It holds for the mobilities that $U_L > U_S > U_T > U_I$. The concentration profiles of the components during the separation are given in Fig. 2a.

During the separation the mixed zone of the leading electrolyte and the impurity remains preserved and the concentration ratio, $c_L/c_I$, is constant during the whole separation. A new zone is formed of the sample and the impurity and the concentration ratio, $c_S/c_I$, as well as the mixed zone length are constant. The impurity gradually penetrates into the terminating electrolyte and forms a mixed region with it. The concentration ratio, $c_T/c_I$, varies with time and changes with the position in the capillary.

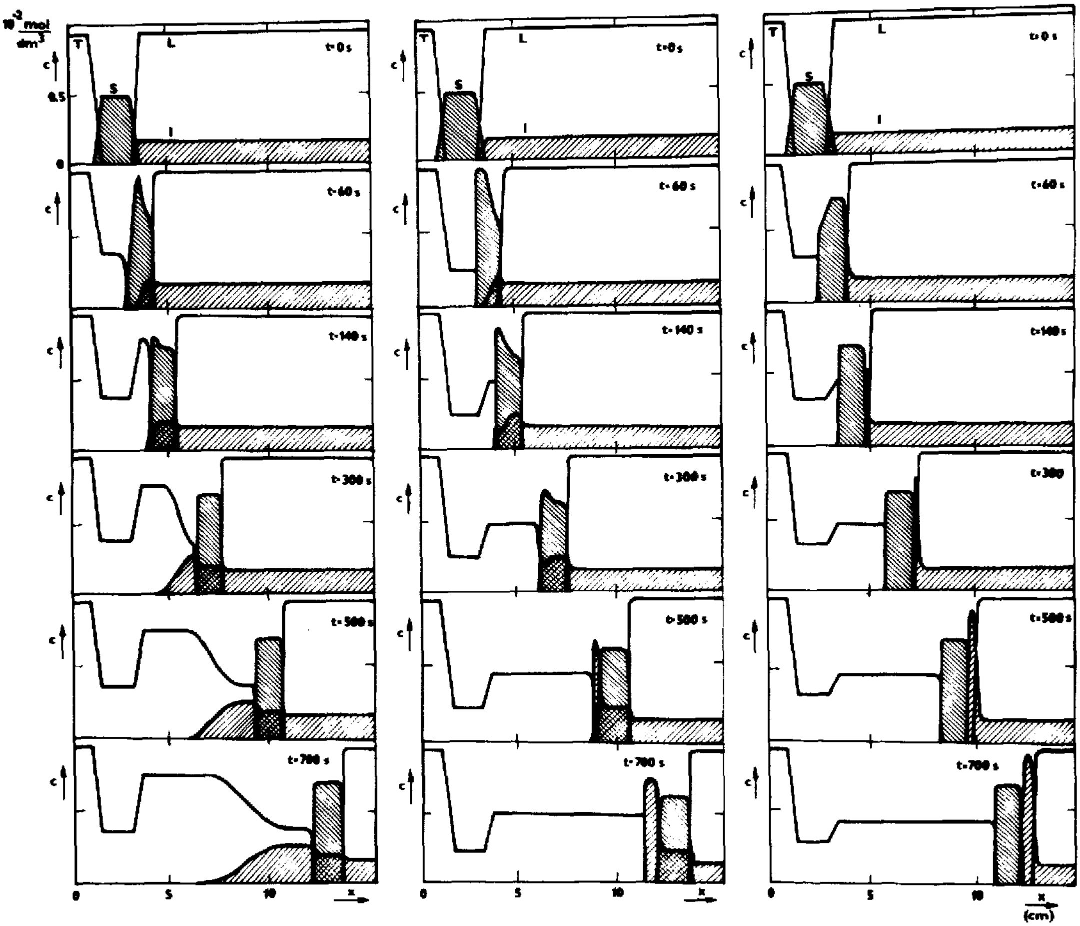
(b) Impurity I is electrolyte 3, leading electrolyte L is electrolyte 1, terminating electrolyte T is electrolyte 4 and sample S is electrolyte 2. It holds for the mobilities that $U_L > U_S > U_I > U_T$. The concentration profiles of the components during the separation are given in Fig. 2b.

During the separation the mixed zone of the leading electrolyte and the impurity is preserved and the concentration ratio, $c_L/c_I$, is constant during the whole separation. A new fixed zone is formed of the sample and the impurity, with the concentration ratio, $c_S/c_I$, and the mixed zone length constant. The impurity gradually forms and independent zone between the sample and the terminating electrolyte. The concentration $c_I$ in this zone is constant and the zone length increases with increasing time. The terminating electrolyte remains pure during the whole separation.

(c) Impurity I is electrolyte 2, leading electrolyte L is electrolyte 1, terminating electrolyte T is electrolyte 4 and sample S is electrolyte 3. It holds for the mobilities that $U_L > U_I > U_S > U_T$. The concentration profiles of the components during the separation are given in Fig. 2c.

During the separation, the mixed zone of the leading electrolyte and the impurity is preserved and the concentration ratio, $c_L/c_I$, is constant. The impurity gradually forms an independent zone between the leading electrolyte and the sample. The concentration $c_I$ in this zone is constant and the zone length increases with increasing time. The sample and terminating electrolyte zones remain pure during the whole separation.

In the bottom part of Fig. 2a–c, the course of the conductance $G$ along the capillary after 700 sec of the separation is given. The situation is analogous to that for impurities in the terminating electrolyte, *i.e.*, the identification of the composition of the individual zones on the sole basis of the conductivity detector recording may not be reliable. The concentration profile of the common counter ion is not given.



The course of the conductance along the capillary

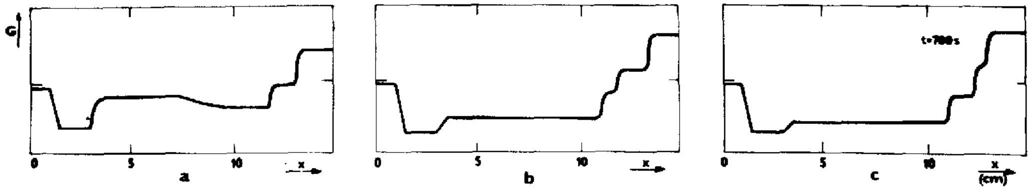


Fig. 2. Separation dynamics for impurities present in the leading electrolyte.

*Sample injected through a valve into the free space between the leading and terminating electrolytes*

With correct sampling by this method, the sample is virtually not mixed with the leading and terminating electrolyte at the beginning of the separation. During the separation, the boundaries of the leading and terminating electrolytes are only sharpened and the sample is gradually separated into its components. The separation is illustrated in Fig. 3a.

Fig. 3. Dynamics of the isotachophoretics separation for: (a) sampling through a valve into the free space between the leading and terminating electrolyte; (b) sampling with a syringe before the boundary into the terminating electrolyte; (c) sampling with a syringe behind the boundary into the leading electrolyte.

### *Sample injected with a syringe before the boundary into the terminating electrolyte and forming a mixed zone with the terminating electrolyte*

This sampling method is equivalent to the case when the terminating electrolyte ions are present in the sample injected through a sampling valve. Here also the individual sample components form independent zones at the beginning of the separation, but the ions of the terminating electrolyte are also simultaneously separated. A steady state is again attained, as can be seen in Fig. 3b.

### *Sample injected with a syringe behind the boundary into the leading electrolyte and forming a mixed zone with the leading electrolyte*

This sampling method is equivalent to the case when the ions of the leading electrolyte are present in the sample injected through a sampling valve. The individual sample components are again separated into independent zones at the beginning of the separation and the ions of the leading electrolyte are also separated. A steady state is again attained, as shown in Fig. 3c.

### *Sample injected with a syringe into the boundary between the leading and terminating electrolytes*

If the injection is carried out accurately, the sample is only partially washed out into the leading and terminating electrolytes. With inaccurate injection, a mixed zone of the sample with the leading and terminating electrolytes is formed. The following two cases were therefore studied.

(a) The sample forms a mixed zone with the leading and terminating electrolytes on injecting with a syringe. Even in this instance the mixture formed (the sample plus the cations of the leading and the terminating electrolytes) is gradually separated into the individual ions according to the laws of isotachophoretic separation, the individual boundaries are sharpened and separated zones of the individual sample components are formed, as can be seen in Fig. 4a.

(b) The sample is partially washed out into both the leading and terminating electrolytes during injection. Even if the washing out into the leading and terminating electrolytes extends over a relatively large part of the capillary length, the individual sample components are eventually separated, as illustrated in Fig. 4b.

## CONCLUSION

In none of the above instances of the presence of impurities in the terminating or leading electrolyte is a steady state established during the separation, so this is not isotachophoresis in the true sense of the word.

The course of the conductance along the capillary in the given time (or the time course of the conductance at a given position in the capillary derived from it, *i.e.*, the conductivity detector response) does not permit a differentiation between zones of pure components and mixed zones (see Figs. 1b and c and 2b and c). Therefore, the only reliable solution of the problem of impurities is to carry out of a control experiment, *e.g.*, checking of the shape of the boundary between the leading and terminating electrolytes in a separation carried out under the same conditions but in the absence of the sample.

When the conductivity detector signal slopes in the region of the leading (ter-

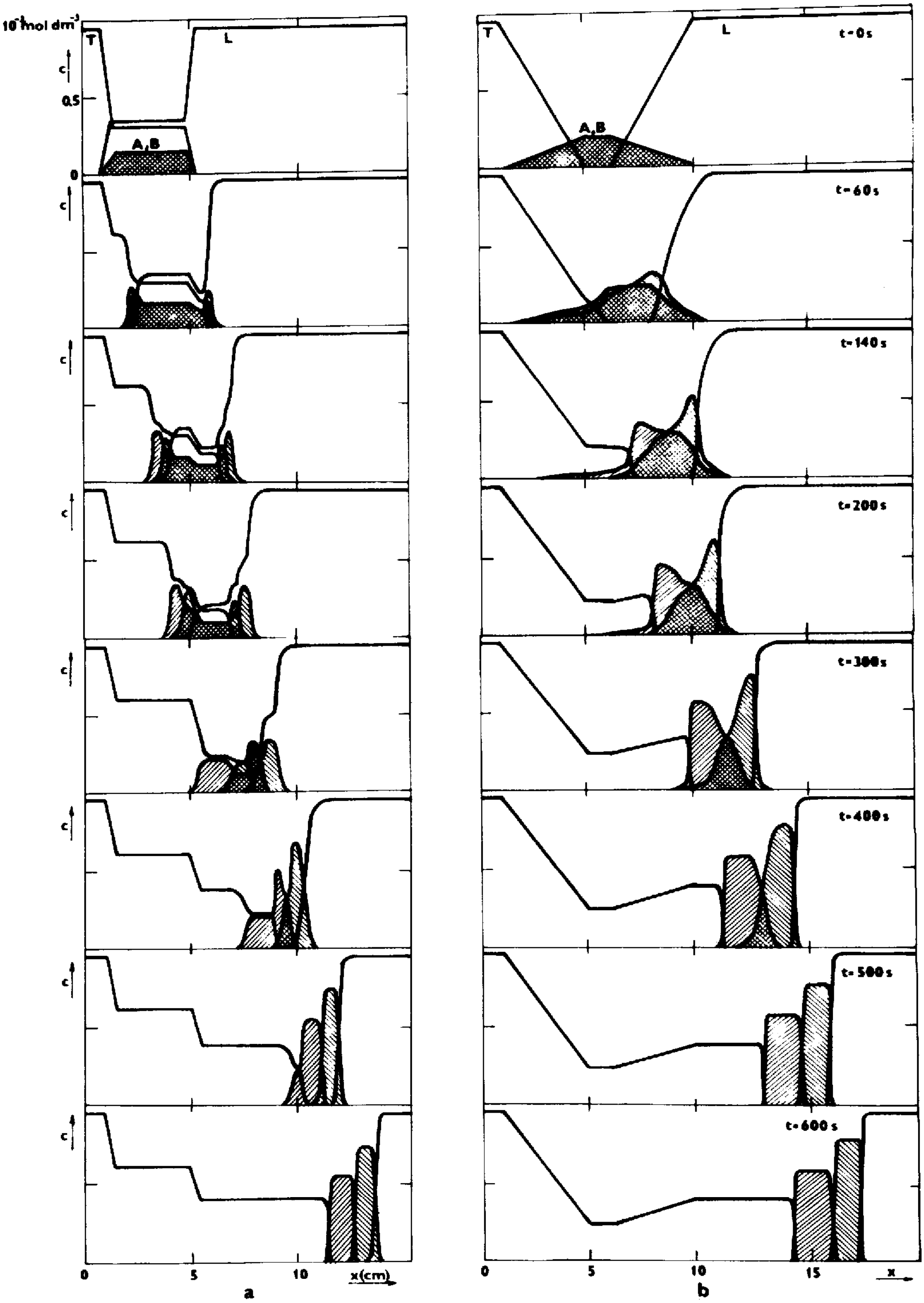


Fig. 4. Dynamics of the isotachophoretic separation for when (a) the sample forms a mixed zone with the leading and terminating electrolyte and (b) the sample is partially washed out into the leading and terminating electrolytes.

minating) electrolyte, the fastest impurity in the terminating (the slowest impurity in the leading) electrolyte may be present; this is analogous to the situation depicted in Figs. 1a and 2a.

It can be concluded from the study of the dependence of the dynamics of isotachophoretic separations on the sample injection method that in the separation of the cations (anions) of strong electrolytes (when the mobility, $U$, of the ions is virtually independent of pH), a steady state is always attained and pure zones of the leading and terminating electrolytes and of the sample components are formed, independently of the sampling method; concentrations $c_L$, $c_A$, $c_B$ and $c_T$ and the lengths of the zones of A and B are constant after establishment of the steady state, in agreement with the Kohlrausch regulation function. Also, with substantial washing out of the sample into the leading and terminating electrolytes, a relatively large portion of the capillary and thus also a longer experimental time are required for attainment of the steady state.

## REFERENCE

1 V. Fidler, J. Vacík and Z. Fidler, *J. Chromatogr.*, 320 (1985) 167–174.